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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Kathleen C.M. Campbell
Art Unit 1614
Serial No. 09/911,195
Filed July 23, 2001
Confirmation No. 2942
For THERAPEUTIC USE OF D-METHIONINE TO REDUCE THE TOXICITY OF NOISE
Examiner Jerome D. Goldberg

DECLARATION OF KATHLEEN C. M. CAMPBELL UNDER 37 CFR 1.132

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS,

SIR:

I, Kathleen C.M. Campbell, hereby declare and state as follows:

1. I reside at 11941 Clearspring Drive, Glenarm, Illinois 62536.

2. I received a Doctor of Philosophy in Audiology/Hearing Science from the University of Iowa in 1989.

3. I am currently a Professor and the Director of Audiology Research in the Division of Otolaryngology, Department of Surgery at the Southern Illinois University School of Medicine in Springfield, Illinois; as well as an adjunct professor of Gerontology at Sangamon State University/University of Illinois-Springfield.

4. I am the named inventor of the subject application, which claims methods for treating or preventing ototoxicity in a patient exposed to noise for a time and at an intensity sufficient to result in ototoxicity.

5. I am a co-author of Campbell et al., "D-Methionine provides excellent protection from cisplatin ototoxicity in the rat," Hearing Research, 102, 90-98 (1996), which the Office has cited against the claims of the subject application.

6. I have reviewed the Office actions dated January 22, 2003; July 15, 2003; and September 29, 2003 in the subject application and studied the disclosure of the Campbell et al. reference.

7. I am providing this Declaration to address whether the Campbell et al. reference would have motivated one skilled in the art at the time the claimed invention was made to employ methionine for the prevention or treatment of ototoxicity in a patient exposed to noise for a time and at an intensity sufficient to result in ototoxicity. After reviewing the Campbell et al. reference and the comments made in the Office actions referenced above, it is my opinion that the Campbell et al. reference does not provide the necessary motivation to lead one of ordinary skill in the art to the subject matter of the invention defined by claims 1 and 3-41.

8. The Campbell et al. reference describes the administration of D-methionine to male rats for the prevention of ototoxicity caused by cisplatin, a chemotherapeutic agent having known ototoxic effects. The reference is entirely devoid of any mention or implication that D-methionine could or would protect against noise-induced hearing loss. Significantly, the reference discloses that D-methionine protected not only against cisplatin-induced hearing loss but also against cisplatin-induced nephrotoxicity and weight loss, neither of which are factors in noise-induced

ototoxicity, and which therefore imply different mechanisms of toxicity and protection.

9. The Campbell reference includes some analysis as to the possible mechanisms by which cisplatin ("CDDP") causes ototoxicity, nephrotoxicity and weight loss, and the manner in which D-methionine may counter the effect of CDDP. Nothing in the reference suggests that noise causes hearing loss by a mechanism in any way similar to the effect of CDDP. In fact, the reference says nothing about noise-induced ototoxicity.

10. Having conducted substantial research in the field of ototoxicity, I am familiar with the literature relating to ototoxicity as resulting from various causes, including CDDP and noise. There are substantial differences in the mechanisms by which hearing loss is induced by noise and by which hearing loss is induced by cisplatin. The differences in mechanism between hearing loss induced by noise and ototoxicity caused by administration of CDDP are evidenced by changes in cells of the ear upon exposure to these agents. These differences are evident in both the stria vascularis and the organ of Corti. In the stria vascularis, cisplatin causes intracellular edema with bulging and compressed marginal cells along the lumen (Meech et al 1997, Campbell et al 1999), but noise causes extracellular strial edema with gaps between the marginal cells at the lumen (Duvall et al 1974, Lipscomb et al 1977). Further, in strial marginal cells, cisplatin causes migration of mitochondria from the subnuclear to the supranuclear sections of the marginal cells but without an overall loss of numbers of mitochondria (Meech et al 1997, Campbell et al 1999). Noise damage does not cause mitochondrial migration in the

marginal cells but can decrease the number of mitochondria (Johnsson and Hawkins (1972)). For marginal cell nuclei, cisplatin can cause degradation of the nuclear envelope and a light appearance of the marginal cell nuclei with apparent loss of chromatin and sometimes missing nucleolus (Meech et al 1997, Campbell et al 1999). In contrast, noise exposure can cause pyknotic marginal cell nuclei with increased amounts of chromatin (Johnsson and Hawkins 1972 Duvall et al 1974). Possibly the most remarkable difference is that the most commonly noted changes to the stria vascularis following noise exposure in animals are a variety of vascular changes (Johnsson and Hawkins 1972, Duvall et al 1974, Lipscomb et al 1977, Santi and Duvall 1978, Vertes et al 1979, Prazma et al 1983, Shaddock et al 1984). Further, some studies have hypothesized that outer hair cell loss and hearing loss due to noise are, at least in part, a consequence of strial vascular changes (Seidman et al 1993). However, vascular changes in the stria vascularis have not been reported as a result of cisplatin exposure. Other differences exist in Reissner's membrane and the reticular lamina. Noise can rupture Reissner's membrane (Duvall et al 1974) and can rupture or cause holes in the reticular lamina (Duvall et al. 1974, Voldrich and Ulehova 1980, Bohne et al. 1984). These types of damage could result in a mixing of perilymphatic and endolymphatic fluids with direct chemical toxicity to surrounding cells. However, these two types of damage have never been reported as a consequence of cisplatin exposure.

11. In addition, the different ototoxicities exhibit different clinical behaviors. Cisplatin can induce systemic toxicity including neurotoxicity, gastrointestinal toxicity, and peripheral neuropathy in addition to hearing loss, while

high-level noise exposure specifically causes hearing loss. Cisplatin can induce delayed hearing loss which arises long after exposure, sometimes after a period of months; and the hearing loss is almost always irreversible. High-level noise is known to cause both temporary threshold shift and permanent threshold shift. However, unlike cisplatin, noise does not induce hearing loss that is first manifested months after the exposure.

12. As discussed in the Campbell et al. reference, the mechanism by which D-methionine is believed to protect against cisplatin-induced ototoxicity teaches away from predicting any otoprotective action against noise-induced ototoxicity. The Campbell et al. reference reports that D-methionine most likely protects against cisplatin-induced ototoxicity by binding to the cisplatin:

> It is logical that free D-Met may preferentially bind to CDDP [i.e., cisplatin] because of the steric hindrance of the protein bound sulfur groups. This protection could occur by preferential binding of the CDDP to D-Met or perhaps D-Met could reverse the Pt binding to the protein bound methionine and glutathione as do other sulfur containing compounds. . . . D-Met binding to CDDP may also protect free L-methionine (L-Met), an essential amino acid. (Campbell et al., p. 95, col. 1.)

13. One skilled in the art would not be led by the Campbell et al. reference to try D-methionine for treating noise-induced ototoxicity because one skilled in the art would not necessarily infer that a treatment effective against cisplatin-induced ototoxicity would also be effective for treating noise-induced ototoxicity. For example, although sodium thiosulfate, fosfomycin, and diethyldithiocarbamate have been shown to protect against cisplatin-induced hearing loss in animals, so far as I am aware none of these agents

has ever been reported or apparently even investigated as protecting against noise-induced hearing loss. Undoubtedly if one of these agents had been found to protect against noise-induced hearing loss, the findings would have been published and would probably have received much attention in the literature, because such findings would have potentially significant clinical impact. If an agent's otoprotection against cisplatin-induced hearing loss motivated one skilled in the art to use that agent to protect against noise-induced hearing loss, it would be logical that all of sodium thiosulfate, fosfomycin, and diethyldithiocarbamate would at least have been tested for that purpose. Based on my familiarity with the field, I would almost certainly be knowledgeable of any testing of these agents for such purpose. Moreover, so far as I am aware, no evidence of testing exists, which supports the conclusion that one skilled in the art would have been motivated by evidence of D-methionine's protection against cisplatin-induced ototoxicity to use D-methionine to achieve otoprotection against noise-induced ototoxicity.

14. U.S. provisional application serial no. 60/069,761 (Kopke et al.) teaches prevention or reversal of hearing loss induced by cisplatin and noise through support of the inner ear's antioxidant defenses by

> increasing antioxidant enzyme levels in the inner ear through the application of agents such as the adenosine agonist R-PIA or other similar agents, or through the application of antiapoptotic agents or trophic factors (growth factors) which may also upregulate antioxidant enzyme levels. (page 7, lines 14-16)

In addition, Kopke et al. teach that an increase in antioxidant levels in the inner ear is "aimed at increasing inner ear glutathione (GSH) levels." (page 7, lines 17-18)

Moreover, the provisional application teaches that various sulfur compounds (e.g. L-2-oxothiazolidine-4-carboxylic acid (OTC), L-N-acetylcysteine (L-NAC), methionine and S-adenosyl-L-methionine (SAMe)) can act as substrates for GSH synthesis when in combination with an agent which upregulates the antioxidant enzyme activity such as R-PIA. (page 7, line 20 to page 8, line 6) Thus, Kopke et al. teach an agent which upregulates the antioxidant enzyme in combination with a sulfur compound which is a substrate for GSH synthesis. However, the claims of the subject application require an "effective amount of an otoprotective agent comprising a compound containing a methionine or a methionine-like moiety." Kopke et al. teach that methionine is merely a substrate for GSH synthesis and do not teach that methionine can be administered in an amount that is effective to provide a protective or rescue effect on the inner ear when exposed to a toxic or traumatic insult.

15. Furthermore, of the two other most promising agents for protection from noise-induced hearing loss, one (acetyl-L-carnitine) to my knowledge has never been reported as protecting against cisplatin-induced hearing loss (and therefore was probably not tested for that use) and the other (N-acetyl cysteine (NAC)) has not been demonstrated to be effective against cisplatin ototoxicity. Only one study has addressed *in vivo* testing, but NAC was only one component of an anti-oxidant mixture comprising alpha-tocopherol acid succinate, ascorbic acid, glutathione and N-acetyl cysteine. Alpha-tocopherol acid succinate, and ascorbic acid, have each independently been shown to be protective against cisplatin-induced ototoxicity. Consequently, it cannot be determined if NAC itself contributed to any otoprotection observed. No studies in the

literature have tested NAC alone as an otoprotective agent in an *in vivo* model. *In vitro* studies have produced variable results but the results of *in vitro* studies must be interpreted with caution. Because NAC is a sulfur containing nucleophile, *in vitro* studies could allow NAC to directly interact with the cisplatin, and thus deactivate the cisplatin prior to cellular uptake, a mechanism that may be irrelevant *in vivo* or if it did occur *in vivo* could cause significant anti-tumor interference prohibiting clinical relevancy. No studies actually testing whether or not NAC inhibits cisplatin anti-tumor activity are in the literature. Additionally, NAC has been shown to exacerbate aminoglycoside-induced ototoxicity, (Block et al., 1983). These teachings further demonstrate that an agent's protective action against hearing loss caused by one type of stressor cannot necessarily be used to predict whether it will reduce or exacerbate hearing loss secondary to a different cause. Although as stated above, U.S. provisional application serial no. 60/069,761 (Kopke et al.) teaches that L-NAC is a substrate for GSH synthesis, taken in context, Kopke et al. teach the combination of a substrate for GSH synthesis and an agent which upregulates the antioxidant enzyme activity. In addition, Kopke et al. do not specify whether the combination is specific for hearing loss induced by cisplatin or hearing loss induced by noise. Accordingly, a person of ordinary skill would have discounted the teachings of Kopke et al. with respect to L-NAC due to their knowledge of the art that showed NAC has not been demonstrated to be effective against cisplatin-induced ototoxicity and exacerbated aminoglycoside-induced ototoxicity.

16. The Campbell et al. reference does not mention or imply that D-methionine could or would protect against noise-induced ototoxicity. As discussed above, the Campbell et al. reference describes the administration of D-methionine for the treatment or prevention of cisplatin-induced ototoxicity in male rats receiving chemotherapeutic doses of cisplatin. To evaluate the effects of the D-methionine, hearing tests were administered to the rats. These hearing tests involved the use of tone-burst stimuli and auditory brainstem testing (ABR) threshold shift analysis, which are standard diagnostic hearing tests well-known to those skilled in the art. The Office appears to suggest that the administration of these standard hearing tests is evidence that D-methionine protects against noise-induced ototoxicity:

> Applicant states that the Campbell et al. reference does not contain ototoxicity caused by exposure to noise. This is not correct since the reference contains 'toneburst stimuli' and the instant claims are directed to 'preventing' the condition. (See Paper 9, page 2.)

17. The use of tone-burst stimuli or ABR threshold shift analysis for the purpose of assessing auditory threshold does not constitute toxic noise exposure. While these tests do expose the subject to noise, the tests fall well short of the claimed limitation requiring "exposure to noise <u>for a time and at an intensity sufficient to result in ototoxicity</u>." In fact, the types of stimuli and equipment used to assess hearing threshold in the animal studies reported in the Campbell et al. reference are the same stimuli and equipment used clinically to assess hearing in human infants. The stimuli used in the Campbell et al. reference <u>could not possibly have caused any hearing loss</u>. Indeed, if clinical audiology test equipment for hearing

testing caused hearing loss it would not be used and would not have been approved by the FDA.

18. In the ABR threshold tests described in the Campbell et al. reference, most of the signals used were at very low or near-threshold levels with intensities ranging from 100 dB sound pressure level (SPL) to 0 dB SPL, with a duration of less than 2 milliseconds. The Occupational Safety and Health Administration (OSHA) has clear guidelines regarding what noise exposures constitute risk of hearing loss. According to OSHA guidelines, workers can be legally and safely exposed to noise exposures of 100 dB dBA up to 2 hours per day, 5 days per week for 40 years. *See* US Department of Labor-Occupational Safety and Health Administration, Occupational Noise Exposure: Hearing Conservation Amendment; Final Rule, *Federal Register* 48(46):9738-9784 (1983). The OSHA guidelines are based on a weighted SPL, giving full weight to frequencies between 700 and 9000 Hz but much less weight to frequencies above and below that range. Thus the OSHA guidelines allow significantly more noise spectrally for the same decibel level used as tone-burst stimuli in the Campbell et al. reference. There is not even a remote possibility that OSHA-defined noise exposure risk levels were reached in the auditory tests mentioned in the Campbell et al. reference.

19. I hereby declare and state that all statements made herein are to my own knowledge true; and that all statements made on information and beliefs are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such

willful false statements will jeopardize the validity of the above-identified application or any patent issued thereon.

Bock GR, et al., "Effects of N-acetylcysteine on Kanamycin Ototoxicity in the Guinea Pig," Hearing Research, 9(3):255-62, 1983.

Bohne B and Rabbitt KD (1983) Holes in the Reticular Lamina after Noise Exposure: Implication for Continuing Damage in the Organ of Corti. Hear. Res. 11(1) 41-53

Campbell KCM, Meech RP, Rybak LP, Hughes LF. (1999). D-methionine protects against cisplatin damage to the stria vascularis. *Hear. Res.* 138:13-28.

Campbell KCM, Rybak LP, Meech RP, Hughes LF. (1996). D-Methionine Provides Excellent Protection from Cisplatin Ototoxicity in the Rat. *Hearing Research* 102:90-98.

Duvall, AJ, Ward WD, Lauhala KE (1974) Stria Ultrastructure and Vessel Transport in Acoustic Trauma. Ann Otol 83: 498-514

Johnsson LG and Hawkins JE (1972) Strial Atrophy in Clinical and Experimental Deafness. Laryngoscope 82(7): 1105-25

Lipscomb DM , Axelsson A, Vertes D, Roettger R and Carroll J (1977) The Effect of High Level Sound on Hearing Sensitivity, Cochlear Sensorineuroepithelium and Vasculature of the Chinchilla. Acta Otolaryngol 84:44-56

Meech R, Campbell KCM, Hughes LF Rybak LP. (1998). A Semiquantitative Analysis of the Effects of Cisplatin on the Rat Stria Vascularis. *Hear. Res.* 124:44-59.

Prazma J, Rodgers GK, Pillsbury HC (1983) Cochlear Blood Flow: Effect of Noise Arch Otolaryngol 109:611-615

Santi PA, Duvall AJ (1978) Stria Vascularis Pathology and Recovery Following Noise Exposure. ORL 354-361

Seidman MD, Shivapuja BG and Quirk WS (1993) The Protective Effects of Allopurinol and Superoxide Dismutase on Noise-Induced Hearing Cochlear Damage Otolaryngology-Head and Neck Surgery (6): 1052-1056

Shaddock LC, Hamernik RP, Axelson A (1984) Cochlear Vascular and Sensory Cell Changes Induced by Elevated Temperature and Noise Am J Otol. 5:99-107

Vertes D, Axelson A, Lipscomb DM (1979) Some Vascular Effects of Noise Exposure in the Chinchilla Cochlea. Acta Otolaryngol 88:47-55

Voldrich L, and Ulehlova L (1980) Comparative Method for the Study of Structural Damage in Acoustic Trauma. The Laryngoscope. 90: 1887-1891

Kathleen C.M. Campbell

Date